

09/634,369

=> d his

(FILE 'HOME' ENTERED AT 15:49:22 ON 13 FEB 2003)

FILE 'CAPLUS' ENTERED AT 15:49:39 ON 13 FEB 2003

L1 638 S CELL(P)HYPOXI?(P) (RE(W)OXYGENAT? OR REOXYGENAT?)
L2 1089 S (EET? OR DHET? OR EPOXYEICOSATRIENO? OR DIHYDROXYEICOSATRIENO
L3 2 S L1 AND L2

FILE 'STNGUIDE' ENTERED AT 15:58:00 ON 13 FEB 2003

FILE 'CAPLUS' ENTERED AT 16:07:00 ON 13 FEB 2003

FILE 'STNGUIDE' ENTERED AT 16:10:18 ON 13 FEB 2003

FILE 'STNGUIDE' ENTERED AT 16:20:49 ON 13 FEB 2003

FILE 'CAPLUS' ENTERED AT 16:28:18 ON 13 FEB 2003

L4 532334 S (HETE? OR EPOXYEICOSADIEN? OR EPOXYEICOSAMONO? OR EPOXYEICOSA
L5 532334 S (HETE? OR EPOXYEICOSADIEN? OR EPOXYEICOSAMONO? OR EPOXYEICOSA
L6 14 S L5 AND L1
L7 2806 S (HETE# OR EPOXYEICOSADIEN? OR EPOXYEICOSAMONO? OR EPOXYEICOSA

=> s l7 and l1

L8 1 L7 AND L1

=> d l8 abs ibib kwic 1

L8 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS

AB The role of arachidonate lipoxxygenase activity in **reoxxygenation** induced **cell** injury in adult canine cardiocytes was investigated. The prodn. of hydroxyeicosatetraenoic acids (**HETES**), which are lipoxxygenase metabolites, was measured with high pressure liq. chromatog. in canine cardiac myocytes cultured under **hypoxic** conditions and then **reoxxygenated**. Free radical generation was evaluated by ESR spectroscopy with a spin trapper, 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and luminol enhanced chemiluminescence emission. **Cell** injury was estd. in terms of morphol. changes and release of intracellular enzymes. Morphol. damage to myocytes was quantified in terms of the percentage of hypercontracted "round" cells. The effects of nordihydroguaiaretic acid, AA-861, mepacrine, indomethacin, aspirin, .alpha. tocopherol, and 2-O-octadecylascorbic acid (CV-3611) on lipoxxygenase metab., free radical generation and **cell** injury were also assessed. Cardiac myocytes produced 5-**HETE** and 12-**HETE** at less than 0.1 ng.cntdot.mg-1 protein under normoxic conditions. Prodn. of **HETE** was greatly increased at five hours of **reoxxygenation** after 45 min of **hypoxia** [5-**HETE**=12.0(SEM 0.5), 12-**HETE**=23.6(1.1) ng.cntdot.mg-1 protein]. Both DMPO-OH adduct generation and chemiluminescence emission were considerably increased after one to three hours of **reoxxygenation**, although they increased only slightly after 45 min of **hypoxia**. After five hours of **reoxxygenation**, long rod cells gradually became deformed; 92.0% of the cells were converted to hypercontracted "round" cells. **Cell** injury and **HETE** prodn. were significantly suppressed by nordihydroguaiaretic acid (10 .mu.M), AA-861 (2 .mu.M), and mepacrine (10 .mu.M). Indomethacin (10 .mu.M) and aspirin (50 .mu.M) enhanced **cell** injury and

HETE prodn. .alpha. Tocopherol and CV-3611 greatly suppressed **cell** injury and free radical generation, but not **HETE** prodn. The arachidonate lipoxygenase metabolic pathway may have an important role in **reoxygenation** induced myocardial **cell** injury in adult cardiac myocytes, possibly because of the generation of free radicals.

ACCESSION NUMBER: 1994:160464 CAPLUS
 DOCUMENT NUMBER: 120:160464
 TITLE: Free radical generation coupled with arachidonate lipoxygenase reaction relates to reoxygenation induced myocardial cell injury
 AUTHOR(S): Kuzuya, Tsunehiko; Hoshida, Shiro; Kim, Youngjoon; Oe, Hiroshi; Hori, Masatsugu; Kamada, Takenobu; Tada, Michihiko
 CORPORATE SOURCE: Sch. Med., Osaka Univ., Osaka, Japan
 SOURCE: Cardiovascular Research (1993), 27(6), 1056-60
 CODEN: CVREAU; ISSN: 0008-6363
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The role of arachidonate lipoxygenase activity in **reoxygenation** induced **cell** injury in adult canine cardiac myocytes was investigated. The prodn. of hydroxyeicosatetraenoic acids (**HETEs**), which are lipoxygenase metabolites, was measured with high pressure liq. chromatog. in canine cardiac myocytes cultured under **hypoxic** conditions and then **reoxygenated**. Free radical generation was evaluated by ESR spectroscopy with a spin trapper, 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and luminol enhanced chemiluminescence emission. **Cell** injury was estd. in terms of morphol. changes and release of intracellular enzymes. Morphol. damage to myocytes was quantified in terms of the percentage of hypercontracted "round" cells. The effects of nordihydroguaiaretic acid, AA-861, mepacrine, indomethacin, aspirin, .alpha. tocopherol, and 2-O-octadecylascorbic acid (CV-3611) on lipoxygenase metab., free radical generation and **cell** injury were also assessed. Cardiac myocytes produced 5-**HETE** and 12-**HETE** at less than 0.1 ng.cntdot.mg-1 protein under normoxic conditions. Prodn. of **HETE** was greatly increased at five hours of **reoxxygenation** after 45 min of **hypoxia** [5-**HETE**=12.0(SEM 0.5), 12-**HETE**=23.6(1.1) ng.cntdot.mg-1 protein]. Both DMPO-OH adduct generation and chemiluminescence emission were considerably increased after one to three hours of **reoxxygenation**, although they increased only slightly after 45 min of **hypoxia**. After five hours of **reoxxygenation**, long rod cells gradually became deformed; 92.0% of the cells were converted to hypercontracted "round" cells. **Cell** injury and **HETE** prodn. were significantly suppressed by nordihydroguaiaretic acid (10 .mu.M), AA-861 (2 .mu.M), and mepacrine (10 .mu.M). Indomethacin (10 .mu.M) and aspirin (50 .mu.M) enhanced **cell** injury and **HETE** prodn. .alpha. Tocopherol and CV-3611 greatly suppressed **cell** injury and free radical generation, but not **HETE** prodn. The arachidonate lipoxygenase metabolic pathway may have an important role in **reoxxygenation** induced myocardial **cell** injury in adult cardiac myocytes, possibly because of the generation of free radicals.

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PATENT ASSIGNEE(S): The Brigham and Women's Hospital, Inc., USA; National
Institutes of Health
SOURCE: PCT Int. Appl., 56 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001010438	A1	20010215	WO 2000-US21744	20000810
W: AU, CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1207877	A1	20020529	EP 2000-952688	20000810
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				

PRIORITY APPLN. INFO.: US 1999-148434P P 19990811
US 2000-634369 A2 20000809
WO 2000-US21744 W 20000810

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB **Epoxyeicosatrienoic acids (EETs)** are products of
cytochrome P 450 epoxigenases that have vasodilatory properties similar to
endothelium-derived hyperpolarizing factor (EDHF). The cytochrome P 450
isoform CYP2J2 was cloned and identified as a source of **EETs** in
human endothelial cells. Physiol. concns. of **EETs** or
overexpression of CYP2J2 decreased cytolcine-induced endothelial cell
adhesion mol. expression and prevented subsequent leukocyte adhesion to
the vascular wall by a mechanism involving inhibition of transcription
factor NF-.kappa.B and I.kappa.B kinase (IKK). The inhibitory effects of
EETs were independent of their membrane hypopolarizing effects
suggesting that these mols. play an important non-vasodilatory role in
vascular inflammation.

IT **Hypoxia**, animal

(prevention of cell death from reoxygenation
following; anti-inflammatory actions of cytochrome P 450
epoxygenase-derived eicosanoids in combination with other agents)

IT 97717-69-6D, **Epoxyeicosatrienoic acid**, analogs

RL: BAC (Biological activity or effector, except adverse); BSU (Biological
study, unclassified); MFM (Metabolic formation); THU (Therapeutic use);
BIOL (Biological study); FORM (Formation, nonpreparative); USES (Uses)
(anti-inflammatory actions of cytochrome P 450 epoxygenase-derived
eicosanoids in combination with other agents)

IT 79551-81-8 79551-82-9 81246-84-6 81246-85-7 81276-02-0
81276-03-1 81920-20-9 81943-03-5 97717-69-6,

Epoxyeicosatrienoic acid 218461-95-1, KMR-IV 87-27
325782-17-0, RKB-V 284-24

RL: BAC (Biological activity or effector, except adverse); BSU (Biological
study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES
(Uses)

(anti-inflammatory actions of cytochrome P 450 epoxygenase-derived
eicosanoids in combination with other agents)

=>

```
=> s cell(p)hypoxi?(p) (re(w)oxygenat? or reoxygenat?)
      1618313 CELL
      34729 HYPOXI?
      110862 RE
      36976 OXYGENAT?
      2748 REOXYGENAT?
L1      638 CELL(P)HYPOXI?(P) (RE(W)OXYGENAT? OR REOXYGENAT?)
```

```
=> s (?EET? or ?DHET? or epoxyeicosatrieno? or dihydroxyeicosatrieno?)
'?EET?' NOT LONG ENOUGH FOR LEFT TRUNCATION
```

You have entered a truncated stem whose length is less than the minimum allowed for left truncation in the requested search field. You may increase the length of the stem to the minimum allowed and try again. Enter HELP SFIELDS to find the minimum stem length for left truncation in the requested search field.

```
=> s (?EET? or ?DHET? or epoxyeicosatrieno? or dihydroxyeicosatrieno? or
11,12(2a)EET or 11,12(2a)DHET or 14,15(2a)EET)
```

```
UNMATCHED RIGHT PARENTHESIS 'EET)')
```

The number of right parentheses in a query must be equal to the number of left parentheses.

```
=> s (?EET? or ?DHET? or epoxyeicosatrieno? or dihydroxyeicosatrieno? or
11,12(2a)EET or 11,12(2a)DHET or 14,15(2a)EET)
```

```
'?EET?' NOT LONG ENOUGH FOR LEFT TRUNCATION
```

You have entered a truncated stem whose length is less than the minimum allowed for left truncation in the requested search field. You may increase the length of the stem to the minimum allowed and try again. Enter HELP SFIELDS to find the minimum stem length for left truncation in the requested search field.

```
=> s (EET? or DHET? or epoxyeicosatrieno? or dihydroxyeicosatrieno? or 11,12(2a)EET
or 11,12(2a)DHET or 14,15(2a)EET)
```

```
      813 EET?
      118 DHET?
      458 EPOXYEICOSATRIENO?
      93 DIHYDROXYEICOSATRIENO?
779428 11
1185077 12
      14300 11,12
              (11(W)12)
      530 EET
      92 11,12(2A)EET
779428 11
1185077 12
      14300 11,12
              (11(W)12)
      72 DHET
      12 11,12(2A)DHET
720270 14
1447939 15
      7534 14,15
              (14(W)15)
      530 EET
```

epoxyeicosadien?

epoxyeicosamono?

epoxyeicosano?

HETE X

09/634,369

110 14,15(2A)EET
L2 1089 (EET? OR DHET? OR EPOXYEICOSATRIENO? OR DIHYDROXYEICOSATRIENO?
OR 11,12(2A)EET OR 11,12(2A)DHET OR 14,15(2A)EET)

=> d his

(FILE 'HOME' ENTERED AT 15:49:22 ON 13 FEB 2003)

FILE 'CAPLUS' ENTERED AT 15:49:39 ON 13 FEB 2003

L1 638 S CELL(P)HYPOXI?(P) (RE(W)OXYGENAT? OR REOXYGENAT?)
L2 1089 S (EET? OR DHET? OR EPOXYEICOSATRIENO? OR DIHYDROXYEICOSATRIENO

=> s l1 and l2

L3 2 L1 AND L2

=> d l3 abs ibib kwic 1-2

L3 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS

AB CYP2J2 is abundant in human heart and its arachidonic acid metabolites, the **epoxyeicosatrienoic** acids (**EETs**), have potent vasodilatory, antiinflammatory and cardioprotective properties. This study was designed to examine the role of CYP2J2 in **hypoxia-reoxygenation**-induced injury in cultured bovine aortic endothelial cells (BAECs). Early passage BAECs were exposed to 24-h **hypoxia** followed by 4-h **reoxygenation** (HR). HR resulted in **cell** injury, as indicated by significant increases in lactate dehydrogenase (LDH) release and trypan blue stained cells ($p < 0.01$) and was assocd. with a decrease in CYP2J2 protein expression. Transfection of BAECs with the CYP2J2 cDNA resulted in increased CYP2J2 expression and arachidonic acid epoxigenase activity, compared with cells transfected with an irrelevant green fluorescent protein (GFP) cDNA. HR induced significant injury in GFP-transfected BAECs, as indicated by increases in LDH release and trypan blue-stained cells ($p < 0.01$); however, the HR-induced injury was markedly attenuated in CYP2J2-transfected cells ($p < 0.01$). HR increased cellular 8-iso-prostaglandin F2.alpha. ($p < 0.05$), and decreased eNOS expression, L-arginine uptake and conversion, and nitrite prodn. ($p < 0.01$) in GFP-transfected BAECs. CYP2J2 transfection attenuated the HR-induced increase in 8-iso-prostaglandin F2.alpha. ($p < 0.05$) and decreased the amt. of extracellular superoxide detected by cytochrome c redn. under normoxic conditions ($p < 0.05$) but did not significantly affect HR-induced decreases in eNOS expression, L-arginine uptake and conversion, and nitrite prodn. Treatment of BAECs with synthetic **EETs** and/or epoxide hydrolase inhibitors also showed protective effects against HR injury ($p < 0.05$). These observations suggest: (1) HR results in endothelial injury and decreased CYP2J2 expression; (2) transfection with the CYP2J2 cDNA protects against HR injury; and (3) the cytoprotective effects of CYP2J2 may be mediated, at least in part, by antioxidant effects.

ACCESSION NUMBER: 2001:552438 CAPLUS

DOCUMENT NUMBER: 135:240155

TITLE: Overexpression of cytochrome P450 CYP2J2 protects against hypoxia-reoxygenation injury in cultured bovine aortic endothelial cells

AUTHOR(S): Yang, Baichun; Graham, Lerae; Dikalov, Serguei; Mason, Ronald P.; Falck, John R.; Liao, James K.; Zeldin, Darryl C.

CORPORATE SOURCE: Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of

Health, Research Triangle Park, NC, USA
SOURCE: Molecular Pharmacology (2001), 60(2), 310-320
CODEN: MOPMA3; ISSN: 0026-895X
PUBLISHER: American Society for Pharmacology and Experimental
Therapeutics
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 70 THERE ARE 70 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB CYP2J2 is abundant in human heart and its arachidonic acid metabolites, the **epoxyeicosatrienoic** acids (**EETs**), have potent vasodilatory, antiinflammatory and cardioprotective properties. This study was designed to examine the role of CYP2J2 in **hypoxia-reoxygenation**-induced injury in cultured bovine aortic endothelial cells (BAECs). Early passage BAECs were exposed to 24-h **hypoxia** followed by 4-h **reoxygenation** (HR). HR resulted in **cell** injury, as indicated by significant increases in lactate dehydrogenase (LDH) release and trypan blue stained cells ($p < 0.01$) and was assocd. with a decrease in CYP2J2 protein expression. Transfection of BAECs with the CYP2J2 cDNA resulted in increased CYP2J2 expression and arachidonic acid epoxigenase activity, compared with cells transfected with an irrelevant green fluorescent protein (GFP) cDNA. HR induced significant injury in GFP-transfected BAECs, as indicated by increases in LDH release and trypan blue-stained cells ($p < 0.01$); however, the HR-induced injury was markedly attenuated in CYP2J2-transfected cells ($p < 0.01$). HR increased cellular 8-iso-prostaglandin F2.alpha. ($p < 0.05$), and decreased eNOS expression, L-arginine uptake and conversion, and nitrite prodn. ($p < 0.01$) in GFP-transfected BAECs. CYP2J2 transfection attenuated the HR-induced increase in 8-iso-prostaglandin F2.alpha. ($p < 0.05$) and decreased the amt. of extracellular superoxide detected by cytochrome c redn. under normoxic conditions ($p < 0.05$) but did not significantly affect HR-induced decreases in eNOS expression, L-arginine uptake and conversion, and nitrite prodn. Treatment of BAECs with synthetic **EETs** and/or epoxide hydrolase inhibitors also showed protective effects against HR injury ($p < 0.05$). These observations suggest: (1) HR results in endothelial injury and decreased CYP2J2 expression; (2) transfection with the CYP2J2 cDNA protects against HR injury; and (3) the cytoprotective effects of CYP2J2 may be mediated, at least in part, by antioxidant effects.

L3 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS

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ACCESSION NUMBER: 2001:114981 CAPLUS
DOCUMENT NUMBER: 134:173027
TITLE: Anti-inflammatory actions of cytochrome P450
epoxigenase-derived eicosanoids
INVENTOR(S): Liao, James K.; Zeldin, Darryl